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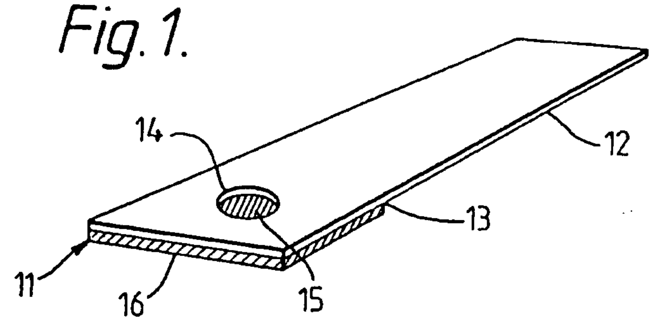
(54) Method for the determination of analytes

(57) A method for determining the presence of an analyte in a fluid is described along with an apparatus specifically designed to carry out the method. the method involves taking a reflectance reading from one surace (15) of an inert porous matrix (11) impregnated with a reagent that will interact with the analyte to produce a light-absorbing reaction product when the fluid being alaysed is applied to the matrix. Reflectance measurements are made at two separate wavelengths in order to eliminate interferences. A timing circuit may be triggered by an initial decrease in reflectance bythe wetting of the surface whose reflectance is being measured by the fluid which passes through the inert matrix. The method and apparatus are particularly suitable for the measurement of glucose levels in blood without requiring separation of red blood cells from serum or plasma.

Fig. 1.



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Description

The present invention relates to a method for the colorimetric determination of chemical and biochemical components (analytes) in whole blood. In one preferred embodiment it concerns a method for colorimetrically measuring the concentration of glucose in whole blood.

The quantification of chemical and biochemical components in whole blood is of ever-increasing importance. Important applications exist in medical diagnosis and treatment and in the quantification of exposure to therapeutic drugs, intoxicants, hazardous chemicals and the like. In some instances, the concentration of materials being determined are either so minuscule - in the range of a microgram or less per deciliter - or so difficult to precisely determine that the apparatus employed is complicated and useful only to skilled laboratory personnel. In this case the results are generally not available for some hours or days after sampling. In other instances, there is often an emphasis on the ability of lay operators to perform the test routinely, quickly and reproducibly outside a laboratory setting with rapid or immediate information display.

One common medical test is the measurement of blood glucose levels by diabetics. Current teaching counsels diabetic patients to measure their blood glucose concentration from two to seven times a day depending on the nature and severity of their individual cases. Based on the observed pattern in the measured glucose concentrations the patient and physician together make adjustments in diet, exercise and insulin intake to better manage the disease. Clearly, this information should be available to the patient immediately.

Currently a method widely used in the United States employs a test article of the type described in U.S. Patent 3,298,789 issued January 17, 1967 to Mast. In this method a sample of fresh, whole blood (typically 20-40 μl) is placed on an ethylcellulose-coated reagent pad containing an enzyme system having glucose oxidase and peroxidase activity. The enzyme system reacts with glucose and releases hydrogen peroxide. The pad also contains an indicator which reacts with the hydrogen peroxide in the presence of peroxidase to give a color proportional in intensity to the sample's glucose concentration.

Another popular blood glucose test method employs similar chemistry but in place of the ethylcellulose-coated pad employs a water-resistant film through which the enzymes and indicator are dispersed. This type of system is disclosed in United States Patent 3,630,957 issued December 28, 1971 to Rey et al.

In both cases the sample is allowed to remain in contact with the reagent pad for a specified time (typically one minute). Then in the first case the blood sample is washed off with a stream of water while in the second case it is wiped off the film. The reagent pad or film is then blotted dry and evaluated. The evaluation is made either by comparing color generated with a color chart or by placing the pad or film in a diffuse reflectance instrument to read a color intensity value.

While the above methods have been used in glucose monitoring for years, they do have certain limitations. The sample size required is rather large for a finger stick test and is difficult to achieve for some people whose capillary blood does not express readily.

In addition, these methods share a limitation with other simple lay-operator colorimetric determinations in that their result is based on an absolute color reading which is in turn related to the absolute extent of reaction between the sample and the test reagents. The fact that the sample must be washed or wiped off the reagent pad after the timed reaction interval requires that the user be ready at the end of the timed interval and wipe or apply a wash stream at the required time. The fact that the reaction is stopped by removing the sample leads to some uncertainty in the result, especially in the hands of the home user. Overwashing can give low results and underwashing can give high results.

Another problem that often exists in simple lay-operator colorimetric determinations is the necessity for initiating a timing sequence when blood is applied to a reagent pad. A user will typically have conducted a finger stick to a obtain a blood sample and will then be required to simultaneously apply the blood from the finger to a reagent pad while initiating a timing circuit with his or her other hand, thereby requiring the use of both hands simultaneously. This is particularly difficult since it is often necessary to insure that the timing circuit is started only when blood is applied to the reagent pad. All of the prior art methods require additional manipulations or additional circuitry to achieve this result. Accordingly, simplification of this aspect of reflectance reading instruments is desirable.

The presence of red blood cells or other colored components often interferes with the measurements of these absolute values thereby calling for exclusion of red blood cells in these two prior methods as they are most widely practised. In the device of U.S. patent 3,298,789 an ethyl cellulose membrane prevents red blood cells from entering the reagent pad. Similarly, the water-resistant film of U.S. patent 3,630,957 prevents red blood cells from entering. In both cases the rinse or wipe also acts to remove these potentially interfering red blood cells prior to measurement.

EP-A-0 110 173 discloses a MBTH-DMAB dry-chemistry porous strip for determining glucose in whole blood samples and how that may be used in an instrument such as a diffuse reflectance spectrophotometer.

The present invention provides a method for measuring the concentration of an analyte in whole blood which comprises:

providing a porous, hydrophilic matrix containing a signal-producing system which is capable of reacting with said analyte to produce a light-absorbtive dye product, said matrix having a first surface, for receiving an unmeasured sample of said whole blood, and a second surface, opposite said first surface, to which at least a portion of said sample can travel through said matrix;
applying said sample to said first surface of said matrix;
allowing at least a portion of said sample to migrate from said first surface to said second surface;
illuminating said second surface of said matrix with light of a first wavelength, which can be absorbed by said light-absorbtive dye product, and light of a second, different wavelength, which can be absorbed by whole blood;
quantitatively measuring light of said first wavelength reflected from said second surface of said matrix after application of said sample without removing excess sample from said first surface to provide a sample reading;
quantitatively measuring light of said second wavelength reflected from said second surface to provide a background reading for correcting said sample reading to account for the absorbance of whole blood; and
calculating a value for the concentration of said analyte in said sample from said sample and background readings.

Preferably the method further comprises the step of quantitatively measuring light reflected from said second surface prior to application of said sample to said matrix to provide a baseline reading, wherein said baseline reading is also used for calculating said value.
Suitably the present method can be performed employing a meter for measuring the concentration of an analyte in whole blood, which is adapted to removably receive a porous, hydrophilic matrix which contains a signal-producing system which is capable of reacting with said analyte to produce a light-absorbtive dye product, said matrix having a first surface, for receiving an unmeasured sample of said whole blood, and a second surface, opposite said first surface, to which at least a portion of said sample can travel through said matrix, said meter comprising:

means for illuminating said second surface of said matrix with light of a first wavelength, which can be absorbed by said light-absorbtive dye product, and light of a second, different wavelength, which can be absorbed by whole blood;
means for detecting and quantitating the light reflected from said second surface of said matrix at said first and second wavelengths;
means for calculating the concentration of said analyte in said sample based on one reading of the reflected light of said first wavelength, corrected for the absorbance of whole blood based on a reading of the reflected light of said second wavelength; and
means for reporting the calculated concentration of said analyte.

Suitably the meter further comprises a self-contained electronic power supply operationally connected to said means for illuminating, means for detecting, means for calculating and means for reporting. For measuring the concentration of glucose in a sample, the said first wavelength is suitably from 625 to 645 nm (which can be absorbed by a dye product produced by the reaction of glucose with the signal-producing system) and said second wavelength is suitably from 690 to 710 nm (which can be absorbed by whole blood). More suitably, said first wavelength is about 635 nm and said second wavelength is about 700 nm.

Preferably the meter further comprises a control means for causing a timing circuit to be initiated on detection of a decrease in the amount of light of either wavelength reflected from said second surface after application of said sample to said first surface of said matrix. Suitably, said control means causes one or more readings of reflected light to be taken at predetermined intervals after detection of said decrease. Preferably said control means is capable of causing said means for detecting to take a reading of light reflected from said second surface of said matrix prior to application of said sample to said first surface of said matrix. More preferably said control means is further capable of causing said means for detecting to collect and store a background detector current reading in the absence of reflected light from said illuminating means.

Preferably, when power is supplied to said control means in an analyte detection mode, said control means automatically causes said means for detecting to read reflected light, to initiate a timing circuit upon detection of said decrease, to collect readings at predetermined intervals after detection of said decrease, to calculate a value for the concentration of analyte in said sample from said readings, and to transfer said value to said reporting means. More preferably, when power is supplied to said control means in a baseline reflectance mode prior to an analyte detection mode, said control means causes said means for detecting to take a baseline reading of the reflected light prior to application of said sample to said matrix and wherein, when said control means is in said analyte detection mode, said baseline reading is also used by said calculating means for calculation of said value.

Suitably the present invention can be carried out using a kit comprising the presently described meter and a porous hydrophilic matrix which contains a signal-producing system which is capable of reacting with said analyte to produce a light absorbtive dye-product, said matrix having a first surface, for receiving an unmeasured sample of said whole

blood, and a second surface, opposite said first surface, to which at least a portion of said sample can travel through said matrix.

Preferably each matrix comprises a polyamide. Suitably the nominal pore size of said matrix is from 0.2 to 1.0 μm.

Preferably the kit is for measuring the concentration of glucose in said whole blood and the signal-producing system comprises 3-methyl-2-benzothiazolinone hydrazone hydrochloride and 3-dimethylamino benzoic acid. Suitably the signal-producing system is at a pH of 3.8 to 5.

The meter may be activated upon a change in reflectance of the matrix when fluid penetrates the matrix. In use the sample of whole blood is added to the matrix which filters out large particles, such as red blood cells, typically with the matrix present in the meter. The signal-producing system produces a dye product which further changes the reflectance of the matrix, which change can be related to the presence of an analyte in a sample.

An exemplary diagnostic assay is the determination of glucose in the whole blood, where the determination is made without interference from the blood and without a complicated protocol subject to use error.

The present application is divided from EP-A-91203031.

The present invention can be more readily understood by reference to the following detailed description when read in conjunction with the attached drawings, wherein:

Figure 1 is a perspective view of one embodiment of a meter suitable for performing the present method containing the reagent matrix to which the fluid being analyzed is applied.

Figure 2 is a block diagram schematic of a meter that can be employed in the practice of the invention.

Figure 3 is a block diagram schematic of an alternate meter that cap be employed in the practice of the invention.

Detailed Description

The Reagent Matrix

The reagent matrix, its various chemical systems and its manner of use are described in EP-A-0 256 806, from which the above mentioned application EP-A-91203031 is divided. However, a very brief description follows with reference to the Figures.

As can be seen from Figure 1, a support holds reagent matrix 11 so that a sample can be applied to one side of the reagent matrix while light reflectance is measured from the side of the reagent matrix opposite the location where sample is applied.

Figure 2 shows a system in which the sample is applied to the side with the hole in the support while light is reflected and measured on the other side of the reagent matrix. Other structures than the one depicted may be employed. The matrix may take various shapes and forms, subject to the limitations provided herein. The matrix will be accessible on at least one surface and usually two surfaces.

The reagent matrix may be attached to the support by any convenient means, e.g. a holder, clamp or adhesives; however, in the preferred mode it is bonded to the support. The bonding can be done with any non-reactive adhesive, by a thermal method in which the support surface is melted enough to entrap some of the material used for the matrix, or by microwave or ultrasonic bonding methods which likewise fuse the matrix to the support. It is important that the bonding be such as to not itself interfere substantially with the diffuse reflectance measurements or the reaction being measured, although this is unlikely to occur as no adhesive need be present at the location where the reading is taken. For example, an adhesive 13 can be applied to the support 12 followed first by punching hole 14 into the combined support and adhesive and then applying matrix 11 to the adhesive in the vicinity of hole 14 so that the peripheral portion of the reagent matrix attaches to the support. The combination of the reagent matrix and the support is hereafter referred to as a strip.

When used with whole blood, the reagent matrix preferably has pores with an average diameter in the range of from about 0.1 to 2.0 μm, preferably about 0.2 to 1.0 and more preferably from about 0.6 to 1.0 μm.

A preferred manner of preparing the reagent matrix is to cast a hydrophilic polymer onto a core of non-woven fibres. The core fibres can be any fibrous material that produces the described integrity and strength, such as polyesters and polyamides. The reagent that will form the light-absorbing reaction product is present within the pores of the matrix but does not block the matrix so that the liquid portion of the whole blood being analyzed can flow through the pores of the matrix, while particles, such as erythrocytes, are held at the surface.

Any signal producing system may be employed that is capable of reacting with the analyte in the sample to produce (either directly or indirectly) a compound that is characteristically absorptive at a wavelength other than a wavelength at which the whole blood substantially absorbs, but a reagent producing a light-absorbtive dye product upon reacting with glucose will be especially useful.

The Measurement Method

The measurement method relies on a change in absorbance, as measured by diffuse reflectance, which is dependent upon the concentration of analyte present in a sample being tested. This change may be determined by measuring the change in the absorbance of the test sample between two or more points in time.

The first step of the method to be considered will be application of the sample to the matrix. In practice, a measurement could be carried out as follows: First a sample of whole blood containing an analyte is obtained. Blood may be obtained by a finger stick, for example. An excess over matrix saturation in the area where reflectance will be measured (i.e. about 5-10 microliters) of whole blood is applied to the reagent matrix. Simultaneous starting of a timer is not required (as is commonly required in the prior art), as will become clear below. Excess fluid can be removed, such as by light blotting, but such removal is also not required. The matrix is typically mounted in a meter for reading light absorbance; e.g. color intensity, by reflectance, prior to application of the sample. Absorbance is measured at certain points in time after application of the sample. Absorbance refers in this application not only to light within the visual wavelength range but also outside the visual wavelength range, such as infrared and ultraviolet radiation. From these measurements of absorbance a rate of color development can be calibrated in terms of analyte concentration.

The Meter

A suitable instrument, such as a diffuse reflectance spectrophotometer with appropriate software, can be made to automatically read reflectance at certain points in time, calculate rate of reflectance change, and, using calibration factors, output the concentration of analyte in the whole blood. Such a meter is schematically shown in Figure 2 wherein a reagent matrix of the invention comprising support 12 to which reagent matrix 11 is affixed is shown. Light source 5, for example a high intensity light emitting diode (LED) projects a beam of light onto the reagent matrix. A substantial portion (at least 25%, preferably at least 35%, and more preferably at least 50%, in the absence of reaction product) of this light is diffusively reflected from the reagent matrix and is detected by light detector 6, for example a phototransistor that produces an output current proportional to the light it receives. Light source 5 and/or detector 6 can be adapted to generate or respond to a particular wavelength light, if desired. The output of detector 6 is passed to amplifier 7, for example, a linear integrated circuit which converts the phototransistor current to a voltage. The output of amplifier 7 can be fed to track and hold circuit 8. This is a combination linear/digital integrated circuit which tracks or follows the analog voltage from amplifier 7 and, upon command from microprocessor 20, locks or holds the voltage at its level at that time. Analog-to-digital converter 19 takes the analog voltage from track and hold circuit 8 and converts it to, for example, a twelve-bit binary digital number upon command of microprocessor 20. Microprocessor 20 can be a digital integrated circuit. It serves the following control functions: 1) timing for the entire system; 2) reading of the output of analog/digital converter 19; 3) together with program and data memory 21, storing data corresponding to the reflectance measured at specified time intervals; 4) calculating analyte concentrations from the stored reflectances; and 5) outputing analyte concentration data to display 22. Memory 21 can be a digital integrated circuit which stores data and the microprocessor operating program. Reporting device 22 can take various hard copy and soft copy forms. Usually it is a visual display, such as a liquid crystal or LED display, but it can also be a tape printer, audible signal, or the like. The instrument also can include a start-stop switch and can provide an audible or visible time output to indicate times for applying samples, taking readings, etc., if desired.

Reflectance Switching

In the present invention, the reflectance circuit itself can be used to initiate timing by measuring a drop in reflectance that occurs when the aqueous portion of the whole blood applied to the reagent matrix migrates to the surface at which reflectance is being measured. Typically, the meter is turned on in a "ready" mode in which reflectance readings are automatically made at closely spaced intervals (typically about 0.2 seconds) from the typically off-white, substantially dry, unreacted reagent matrix. The initial measurement is typically made prior to penetration of the matrix by fluid being analyzed but can be made after the fluid has been applied to a location on the reagent matrix other than where reflectance is being measured. The reflectance value is evaluated by the microprocessor, typically by storing successive values in memory and then comparing each value with the initial unreacted value. When the aqueous portion penetrates the reagent matrix, the drop in reflectance signals the start of the measuring time interval. Drops in reflectance of 5-50% can be used to initiate timing, typically a drop of about 10%. In this simple way there is exact synchronization of aqueous portion reaching the surface from which measurements are taken and initiation of the sequence of readings, with no requirement of activity by the user.

Although the total systems described in this application are particularly directed to the use of polyamide matrices and particularly to the use of such matrices in determining the concentration of various sugars, such as glucose, and

other materials of biological origin, there is no need to limit the reflectance switching aspect of the invention to such matrices. For example, the matrix used with reflectance switching may be formed from any water-insoluble hydrophilic material and any other type of reflectance assay.

Particular Application to Glucose Assay

A particular example with regard to measuring the concentration of glucose in the presence of red blood cells will now be given in order that greater detail and particular advantage can be pointed out. Although this represents a preferred embodiment of the present invention, the invention is not limited to the detection of glucose in blood.

The use of polyamide surfaces to form the reagent matrix provides a number of desirable characteristics. These are that the reagent matrix is hydrophilic (i.e. takes up reagent and sample readily), does not deform on wetting (so as to provide a flat surface for reflectance reading), is compatible with enzymes (in order to impart good shelf stability), takes up a limited sample volume per unit volume of matrix (necessary in order to demonstrate an extended dynamic range of measurements), and shows sufficient wet strength to allow for routine manufacture.

In a typical configuration, the method is carried out using a meter consisting of a plastic holder and the reagent matrix having the signal producing system impregnated therein, as described in EP-A-0 256 806.

The meter used to make the reflectance readings minimally contains a light source, a reflected light detector, an amplifier, an analog to digital converter, a microprocessor with memory and program, and a display device.

The light source typically consists of a light emitting diode (LED). Although it is possible to use a polychromic light source and a light detector capable of measuring at two different wavelengths, a preferred meter would contain two LED sources or a single diode capable of emitting two distinct wavelengths of light. Commercially available LEDs producing the wavelengths of light described as being preferred in the present specification include a Hewlett Packard HLMP-1340 with an emission maximum at 635nm and a Hewlett Packard QEMT-1045 with a narrow-band emission maximum at 700nm. Suitable commercially available light detectors include a Hammamatsu 5874-18K and a Litronix BPX-65.

Although other methods of taking measurements are feasible, the following method has provided the desired results. Readings are taken by the photodetector at specified intervals after timing is initiated. The 635nm LED is powered only during a brief measuring time span that begins approximately 20 seconds after the start time as indicated by reflectance switching. If this reading indicates that a high level of glucose is present in the sample, a 30-second reading is taken and used in the final calculation in order to improve accuracy. Typically, high levels are considered to begin at about 250 mg/dl. The background is corrected with a 700nm reading taken about 15 seconds after the start of the measurement period. The reading from the photodetector is integrated over the interval while the appropriate LED is activated, which is typically less than one second. The raw reflectance readings are then used for calculations performed by the microprocessor after the signal has been amplified and converted to a digital signal. Numerous microprocessors can be used to carry out the calculation. An AIM65 single-board microcomputer manufactured by Rockwell International has proven to be satisfactory.

The present method allows a very simple procedure with minimum operational steps on the pact of the user. In use, a strip is placed in the detector so that the hole in the strip registers with the optics of the meter. A removable cap or other cover is placed over the optics and strip to shield the assembly from ambient light. The measurement sequence is then initiated by pressing a button on the meter that activates the microcomputer to take a measurement of reflected light from the unreacted reagent matrix, called an R_{dry} reading. The cap is then removed and a drop of blood is applied to the reagent matrix, typically while the reagent matrix is registered with the optics and the meter. It is preferred that the strip be left in register with the optics in order to minimize handling. The meter is capable of sensing the application of blood by a decrease in the reflectance when the sample passes through the matrix and reflected light is measured on the opposite side. The decrease in reflectance initiates a timing sequence which is described in detail at other locations in this specification. The cover should be replaced within 15 seconds of sample application, although this time may vary depending on the type of sample being measured. Results are typically displayed at approximately 30 seconds after blood application when a blood glucose sample is being measured, although a 20 second reaction is permissible for blood samples having a concentration of glucose of less than 250mg/dl.

A particularly accurate evaluation of glucose concentration (or any other analyte being measured) can be made using the background current, i.e. the current from the photodetector with power on but with no light reflected from the reagent matrix, in order to make a background correction. It has been demonstrated that over a 2-3 month period that this value does not change for a particular meter, and it is possible to program this background reading into the computer memory as a constant. With a slight modification of the procedure, however, this value can be measured with each analysis for more accurate results. In the modified procedure the meter would be turned on with the lid closed before the strip is in place, and the background current would be measured. The strip would then be inserted into the meter with the cover closed, an R_{dry} measurement taken, and the procedure continued as described above. With this modified procedure the background current does not need to be stable throughout the life of the meter, thereby providing more accurate results.

The raw data necessary for calculating a result in a glucose assay are a background current reported as background reflectance, R_b, as described above; a reading of the unreacted matrix, R_{dry}, also described above; and an endpoint measurement. Using the preferred embodiments described herein, the endpoint is not particularly stable and must be precisely timed from the initial application of blood. However, the meter as described herein performs this timing automatically. For glucose concentrations below 250mg/dl, a suitably stable endpoint is reached in 20 seconds, and a final reflectance, R_{20}, is taken. For glucose concentrations up to 450mg/dl, a 30-second reflectance reading, R_{30}, is adequate. Although the system described herein displays good differentiation up to 800mg/dl of glucose, the measurement is somewhat noisy and inaccurate above 450mg/dl, although not so great as to cause a significant problem. Longer reaction times should provide more suitable readings for the higher levels of glucose concentration.

The 700nm reflectance reading for the dual wavelength measurement is typically taken at 15 seconds (R_{15}). By this time blood will have completely saturated the reagent matrix. Beyond 15 seconds the dye reaction continues to take place and is sensed, to a small part, by a 700nm reading. Accordingly, since dye absorption by the 700nm signal is a disadvantage, readings beyond 15 seconds are ignored in the calculations.

The raw data described above are used to calculate parameters proportional to glucose concentration which can be more easily visualized than reflectance measurements. A logarithmic transformation of reflectance analogous to the relationship between absorbtivity and analyte concentration observed in transmission spectroscopy (Beer's Law) can be used if desired. A simplification of the Kubelka-Monk equations, derived specifically for reflectance spectroscopy, have proven particularly useful. In this derivation K/S is related to analyte concentration with K/S defined by Equation 1.

$$K/S\text{-}t = (1 - R^*t)^2/(2 \times R^*t) \qquad (1)$$

R*t is the reflectivity taken at a particular endpoint time, t, and is the absorbed fraction of the incident light beam described by Equation 2, where R_t is the endpoint reflectance, R_{20} or R_{30}.

$$R^*t = (R_t - R_b)/(R_{dry} - R_b) \qquad (2)$$

R*t varies from 0 for no reflected light (R_b) to 1 for total reflected light (R_{dry}). The use of reflectivity in the calculations greatly simplifies meter design as a highly stable source and a detection circuit become unnecessary since these components are monitored with each R_{dry} and R_b measurement.

For a single wavelength reading K/S can be calculated at 20 seconds (K/S-20) or 30 seconds (K/S-30). The calibration curves relating these parameters to YSI (Yellow Springs Instruments) glucose measurements can be precisely described by the third order polynomial equation outlined in Equation 3.

$$YSI = a_0 + a_1 (K/S) + a_2 (K/S)^2 + a_3 (K/S)^3 \qquad (3)$$

The coefficients for these polynomials are listed in Table 1.

TABLE 1

Coefficients for Third Order Polynomial Fit of Single Wavelength Calibration Curves		
	K/S-20	K/S-30
a_0	-55.75	-55.25
a_1	0.1632	0.1334
a_2	-5.765×10^{-5}	-2.241×10^{-5}
a_3	2.58×10^{-8}	1.20×10^{-8}

The single chemical species being measured in the preferred embodiments is the MBTH-DMAD indamine dye and the complex matrix being analyzed is whole blood distributed on a 0.8µ Posidyne membrane.

The use of the MBTH-DMAB couple allows for correction of hematocrit and degree of oxygenation of blood with a single correction factor. The more typically used benzidine dyes do not permit such a correction. The dye forms a chromophore that absorbs at approximately 635nm but not to any significant extent at 700nm. Slight variations in measuring wavelengths (+/- about 10nm) are permitted. At 700nm both hematocrit and degree of oxygenation can be measured by measuring blood colour.

A review entitled "Application of Near Infra Red Spectrophotometry to the Nondestructive Analysis of Foods : A

Review of Experimental Results", CRC Critical Reviews in Food Science and Nutrition, 18(3) 203-30 (1983), describes the use of instruments based on the measurement of an optical density difference ΔOD (λ_a-λ_b) where $OD\lambda_a$ is the optical density of the wavelength corresponding to the absorption maximum of a component to be determined and $OD\lambda_b$ is the optical density at a wavelength where the same component does not absorb significantly.

The algorithm for dual wavelength measurement is by necessity more complex than for single wavelength measurement but is much more powerful. The first order correction applied by the 700nm reading is a simple subtraction of background color due to blood. In order to make this correction, a relationship between absorbance at 635nm and 700nm due to blood color can be and was determined by measuring blood samples with 0 mg/dl glucose over a wide range of blood color. The color range was constructed by varying hematocrit, and fairly linear relationships were observed. From these lines the K/S-15 at 700nm was normalized to give equivalence to the K/S-30 at 635nm. This relationship is reported in Equation 4, where K/S-15n is the normalized K/S-15 at 700nm.

$$\text{K/S-15n} = (\text{K/S-15} \times 1.54) - 0.133 \tag{4}$$

Note that the equivalence of the normalized 700nm signal and the 635nm signal is only true at zero glucose. The expressions from which the calibration curves were derived are defined by Equations 5 and 6.

$$\text{K/S-20/15} = (\text{K/S-20}) - (\text{K/S-15n}) \tag{5}$$

$$\text{K/S-30/15} = (\text{K/S-30}) - (\text{K/S-15n}) \tag{6}$$

These curves are best fit by fourth-order polynomial equations similar to Equation 3 to which a fourth-order term in K/S is added. The computer-fit coefficients for these equations are listed in Table 2.

TABLE 2

Coefficients for Fourth-Order Polynomial Fit of Dual Wavelength Calibration Curves		
	K/S-20/15	K/S-30/15
a_0	-0.1388	1.099
a_1	0.1064	0.05235
a_2	6.259×10^{-5}	1.229×10^{-4}
a_3	-6.12×10^{-8}	-5.83×10^{-8}
a_4	3.21×10^{-11}	1.30×10^{-11}

A second order correction to eliminate errors due to chromatography effects has also been developed. Low hematocrit samples have characteristically low 700nm readings compared to higher hematocrit samples with the same 635nm reading. When the ratio of (K/S-30)/(K/S-15) is plotted versus K/S-30 over a wide range of hematocrits and glucose concentrations, the resulting line on the graph indicates the border between samples which display chromatography effects (above the curve) and those that do not (below the curve). The K/S-30 for the samples above the curve are corrected by elevating the reading to correspond to a point on the curve with the same (K/S-30)/(K/S-15).

The correction factors reported above were tailor made to fit a single meter and a limited number of reagent preparations. The algorithm can be optimized for an individual meter and reagent in the same manner that is described above.

In summary, the system of the present invention minimizes operator actions and provides numerous advantages over prior art reflectance-reading methods. When compared to prior methods for determining glucose in blood, for example, there are several apparent advantages. First, the amount of sample required to saturate the thin reagent matrix is small (typically 5-10 microliters). Second, operator time required is only that necessary to apply the sample to the thin reagent matrix and close the cover (typically 4-7 seconds). Third, no simultaneous timing start is required. Fourth, whole blood can be used. The method does not require any separation or utilization of red-cell-free samples.

The invention now being generally described, the same will be better understood by reference to the following specific examples which are presented for purposes of illustration only and are not to be considered limiting of the invention unless so specified.

Example I

Reproducibility:

One male blood sample (JG, hematocrit = 45) was used to collect the reproducibility data set forth in Tables 3-5.

TABLE 3

Reproducibility of a Single Wavelength MPX System						
YSI (mg/dl)	Average (mg/dl)		S.D. (mg/dl)		%C.V.	
	20 sec.	30 sec.	20 sec.	30 sec.	20 sec.	30 sec.
25	23.1	23.0	2.1	2.04	9.1	9.0
55	53.3	53.2	3.19	3.32	6.0	6.3
101	101	101	3.0	3.3	3.0	3.3
326	326.6	327	13.3	9.8	4.1	3.0
501		503		17.1		3.4
690		675		28		4.15
810		813		37		4.5

TABLE 4

Reproducibility of a Dual Wavelength MPX System						
YSI (mg/dl)	Average (mg/dl)		S.D. (mg/dl)		%C.V.	
	20 sec.	30 sec.	20 sec.	30 sec.	20 sec.	30 sec.
25	25	27	1.34	1.55	5.4	5.7
55	55	57.4	2.58	2.62	4.7	4.6
101	101	101.5	2.55	2.18	2.5	2.1
326	332	330	15.0	7.1	4.5	2.1
501		505		21.3		4.2
690		687		22.8		3.3
810		817		30.4		3.7

TABLE 5

Reproducibility of a 3.0mm Diameter Aperture		
YSI (mg/dl)	% C.V.	
	4.7mm	3.0mm
55-100	4.8	4.9
300	3.0	5.0
600	3.8	5.5
avg.	$\overline{3.9}$	$\overline{5.1}$

The blood was divided into aliquots and spiked with glucose across a range of 25-800mg/dl. Twenty determinations were made at each glucose test concentration from strips taken at random from a 500 strip sample (Lot FJ4-49B). The results of this study lead to the following conclusions:

1. Single vs. Dual Wavelength: The average C.V. for the 30-second dual result was 3.7% vs. 4.8% for the 30-second single wavelength result, an improvement of 23% across a glucose range of 25-810mg/dl. There was a 33% improvement in C.V. in the 25-326mg/dl glucose range. Here the C.V. decreased from 5.4% to 3.6%, a significant improvement in the most used range. The 20-second dual wavelength measurement gave similar improvements in C.V. compared to the single wavelength measurement in the 25-325mg/dl range (Tables 3 and 4).

2. Dual Wavelength, 20 vs. 30-second Result: The average C.V. for a 20-second result in the 25-100 mg/dl range is nearly identical to the 30-second reading, 4.2% vs. 4.1%. However, at 326 mg/dl the 30-second reading has a C.V. of 2.1% and the 20-second result a C.V. of 4.5%. As was seen in the K/S-20 response curve, the slope begins to decrease sharply above 250 mg/dl. This leads to poor reproducibility at glucose concentrations greater than 300 mg/dl for the 20-second result. From this reproducibility data the cutoff for the 20-second result is somewhere between 100 and 326 mg/dl. A cutoff of 250 mg/dl was later determined from the results of the recovery study set forth in Example II.

3. Aperture Size: A smaller optics aperture size, 3.0 mm vs. 5.0 mm., was investigated. Initial experimentation using a 10- replicate, hand-dipped disk sample did show improved C.V.s with the 3.0mm aperture, apparently because of easier registration with the system optics. However, when machine-made roll membrane was used, the average C.V. (Table 5) of the larger aperture, 4.7mm, was 3.9% vs. an average C.V. for the 3.0mm aperture of 5.1%. This 30% increase in C.V. was probably due to the uneven surface of the roll membrane lot as discussed below.

Example II

Recovery:

For comparison of the present method (MPX) against a typical prior art method using a Yellow Springs Instrument Model 23A glucose analyser manufactured by Yellow Springs Instrument Co., Yellow Springs, Ohio (YSI), blood from 36 donors was tested. The donors were divided equally between males and females and ranged in hematocrit from 35 to 55%. The blood samples were used within 30 hours of collection, with lithium heparin as the anti-coagulant. Each blood sample was divided into aliquots and spiked with glucose to give 152 samples in the range of 0-700 mg/dl glucose. Each sample was tested in duplicate for a total of 304 data points.

Response curves were constructed from these data and glucose values then calculated from the appropriate equation (Tables 1 and 2). These MPX glucose values were then plotted vs. the YSI values to give scattergrams.

Comparison of MPX Systems: For both the 20-second and 30-second measurement times there is visually more scatter in the single-wavelength scattergrams than the dual-wavelength scattergrams. The 20-second reading becomes very scattered above 250 mg/dl but the 30-second measurement does not have wide scatter until the glucose level is ≥500 mg/dl.

These scattergrams were quantitated by determining the deviations from YSI at various glucose concentrations. The following results were obtained.

TABLE 6

Accuracy of MPX from Recovery Data					
MPX Wavelength	Measurement Time (sec.)	S.D. (mg/dl)	C.V. for Range*		
		0-50	50-250	250-450	450-70
Single	20	±5.6	7.2	14.5	-
Single	30	±6.9	7.1	8.8	10.2
Dual	20	±2.3	5.3	12.8	-
Dual	30	±2.19	5.5	5.8	8.4

<u>Note</u>: These are inter method C.V.s.
a. The dual wavelength system gave C.V.s that ranged 30% lower than the single wavelength system.
b. The single wavelength system, from 0-50 mg/dl, showed a S.D. of ±6-7 mg/dl whereas the S.D. for a dual wavelength measurement was only ±2.2 mg/dl.
c. The cutoff for a 30-second MPX measurement is 250 mg/dl. For the 50-250 mg/dl range both the 20- and 30-second measurements gave similar inter-method C.V.s (approximately 7% for single wavelength, 5.5% for dual wavelength). However, in the 250-450 mg/dl range inter-method C.V.s more than double for the 20-second reading to 14.5% for the single and 12.8% for the dual wavelength.
d. The 30-second reading was unusable above 450 mg/dl for both the single and dual wavelength measurement (C.V.s of 10.2 and 8.4%).

In conclusion, two MPX systems gave optimum quantitation in the 0-450 mg/dl range.

1. <u>MPX 30 Dual</u>: This dual wavelength system gave a 30-second measurement time with a 95% confidence limit (defined as the probability of a measurement being within 2 S.D. of the YSI) of 11.3% (C.V.) for the range from 50-450 mg/dl (Table 7) and ±4.4 mg/dl (S.D.) for 0-50 mg/dl.

2. <u>MPX 30/20 Dual</u>: This dual wavelength system gave a 20-second measurement time in the 0-250 mg/dl range and a 30-second time for the 250-450 range. The 95% confidence limits are nearly identical to the MPX 30 Dual System (Table 7), 11.1% (C.V.) for 50-450 mg/dl and ±4.6 mg/dl (S.D. for 0-50 mg/dl).

TABLE 7.

Comparison of 95% Confidence Limits for MPX, GlucoScan Plus and Accu-Chek bG* Reagent Strips

Measuring Range mg/dl	MPX Single Wavelength 20 sec.	MPX Single Wavelength 30 sec.	MPX Dual Wavelength 20 sec.	MPX Dual Wavelength 30 sec.
0-50	11.2 mg/dl	13.8 mg/dl	4.6 mg/dl	4.4 mg/dl
50-250	14.4%	14.2%	10.6%	11.0%
250-450	-	17.6%	-	11.6%

77-405	GlucoScan Plus (Drexler Clinical)	15.9%
77-405	Accu-Chek bG (Drexler Clinical)	10.7%
50-450	MPX 20/30 Dual Hybrid	11.1%
50-450	MPX 30 Dual	11.3

* Confidence limits for MPX were from the YSI. The confidence limits for GlucoScan Plus and Accu-Chek bG were from the regression equation vs. YSI which eliminates bias due to small differences in calibration.

Example III

Stability:

Most of the bench-scale work carried out in optimizing stability was completed using hand-dipped 0.8μ Posidyne membrane disks. The specific dye/enzyme formulation was set forth previously.

1. Room Temperature Stability: This study attempted to chart any change in response of the 0.8μ Posidyne membrane reagent stored at 18-20°C over silica gel desiccant. After 2.5 months there was no noticeable change as measured by the response of a room temperature sample vs. the response of a sample stored at 5°C. Each scattergram represented a glucose range of 0-450 mg/dl.

2. Stability at 37°C: A 37°C stability study using the same reagent as the RT study was carried out. The differences in glucose values of reagent stressed at 37°C vs. RT reagent, for matrices stressed with and without adhesive, was plotted over time. Although the data was noisy, due to the poor reproducibility of handmade strips, the stability was excellent for strips whether they were stressed with or without adhesive.

3. Stability at 56°C: Eight 5- to 6-day stability studies were carried out using different preparations of a similar formulation on disk membrane (Table 8). For the low glucose test level (80-100 mg/dl) the average gulcose value dropped upon stressing by 3.4%, with the highest drop being 9.55%. At the high test level (280-320 mg/dl) the glucose reading declined by an average of 3.4%, the largest decline being 10.0%.

TABLE 8

Stability of pH = 4.0, .8 Posidyne Disk Reagent Formulation Stressed for 5 to 6 Days at 56°C		
Sample No.	% Difference (56°C vs. RT Sample)	
	YSI (80-100 mg/dl)	YSI (280-320 mg/dl)
FJ22B	-6.25	+5.4
FJ27A	-4.0	-5.14
FJ28B	-2.4	-5.3
FJ30H	-9.55	-10.0
FJ31C	+4.43	-1.24
FJ36	-3.2	-8.5
FJ48B*	-3.0	0.0
GM48A*	-3.0	-2.5
Average of 8	-3.4	-3.4

* These two samples contained twice the normal concentration of enzyme and dye.

A study of the 56°C stressing of this matrix over a 19-day period showed no major difference for strips stressed with or without adhesive. In both cases the 19-day decline in glucose value was <15% at low test levels (80-100) and 300 mg/dl. Another 56°C study using hand-dipped 0.8μ Posidyne membrane with twice the normal concentration of enzyme and dye was completed. Two separate preparations of the same formulation were made up and the stability measured over a 14-day period. The average results of the two studies were plotted. Changes were within ±10% over the 14-day period at both the high and low glucose test level. These data show this formulation to be particularly stable.

Example IV

Sample Size:

The sample size requirements for MPX are demonstrated in Table 9.

TABLE 9

Effect of Sample Size on MPX Measurements										
Sample Size (ul)	Dual Wavelength Average					Single Wavelength Average				
Low Glucose YSI = 56										
3	41	50	39	31	40	31	42	30	19	30
4	44	49	49	49	48	41	45	44	45	44
5	54	48	49	51	50	50	49	48	49	49
10	48	48	50	47	48	54	53	56	55	54
20	49	49	49	50	49	55	57	58	60	58
High Glucose YSI = 360										
3	301	260	276	286	280	274	232	244	260	252
4	383	378	367	341	367	361	356	342	318	344
5	398	402	382	370	388	378	387	366	351	370
10	364	362	378	368	368	356	358	379	369	366
20	375	370	380	378	376	380	382	389	385	384

The volumes reported in the table were transferred to the reagent matrix shown in Figure 1 using a micro pipet. When blood from a finger stick is applied to a strip the total sample cannot be transferred, therefore the volumes reported here do not represent the total sample size needed to be squeezed from the finger for the analysis. A 3-µl sample is the minimum necessary to completely cover the reagent matrix circle. This does not provide enough sample to completely saturate the reagent matrix and MPX gives low results. A 4-µl sample barely saturates the reagent matrix, while a 5-µl sample is clearly adequate. A 10-µl sample is a large shiny drop and a 20-µl sample is a very large drop and is only likely to be used when blood from a pipet is used for sampling.

At low glucose concentration the single wavelength result has some dependence on sample size which is completely eliminated using the dual wavelength measurement. Although this dependence with the single wavelength might be considered acceptable, it is clearly undesirable.

Example V

Reproducibility:

Experimental measurements described above were always run in replicate, usually 2, 3 or 4 determinations per data point. These sets have always shown close agreement even for samples with extreme hematocrits or extreme oxygen levels. C.V.s were well below 5%. It appears, therefore, that reproducibility is very good to excellent.

The subject invention provides for many advantages over systems which are presently available commercially or have been described in the literature. The protocols are simple and require little technical skill and are relatively free of operator error. The assays can be carried out rapidly and use inexpensive and relatively harmless reagents, important considerations for materials employed in the home. The user obtains results which can be understood and used in conjunction with maintenance therapy. In addition, the reagents have long shelf lives, so that the results obtained will be reliable for long periods of time. The meter which has been discribed and will be employed in the present method is simple and reliable and substantially automatic.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many modifications and changes can be made thereto

Claims

1. A method for measuring the concentration of an analyte in whole blood which comprises:

providing a porous, hydrophilic matrix containing a signal-producing system which is capable of reacting with

said analyte to produce a light-absorbtive dye product, said matrix having a first surface, for receiving an unmeasured sample of said whole blood, and a second surface, opposite said first surface, to which at least a portion of said sample can travel through said matrix;

applying said sample to said first surface of said meter matrix;

allowing at least a portion of said sample to migrate from said first surface to said second surface;

illuminating said second surface of said matrix with light-absorbtive dye product, and light of a second, different wavelength, which can be absorbed by whole blood;

quantitatively measuring light of said first wavelength reflected from said second surface of said matrix after application of said sample without removing excess sample from said first surface to provide a sample reading;

quantitatively measuring light of said second wavelength reflected from said second surface to provide a background reading for correcting said sample reading to account for the absorbance of whole blood; and

calculating a value for the concentration of said analyte in said sample from said sample and background readings.

2. The method of claim 1, which further comprises the step of quantitatively measuring light reflected from said second surface prior to application of said sample to said matrix to provide a baseline reading, wherein said baseline reading is also used to calculate said value.

3. The method of claim 1 or claim 2, for measuring the concentration of glucose in said sample, wherein said first wavelength is from 625 to 645 nm (which can be absorbed by a dye product produced by the reaction of glucose with the signal-producing system) and said second wavelength is from 690 to 710 nm (which can be absorbed by whole blood).

4. The method of claim 3, wherein said first wavelength is about 635 nm and said second wavelength is about 700 nm.

5. The method of any one of claims 1 to 4, further comprising initiating a timing circuit to be initiated on detection of a decrease in the amount of light of either wavelength reflected from said second surface after application of said sample to said first surface of said matrix.

6. The method of claim 5, including initiating one or more readings of reflected light to be taken at predetermined intervals after detection of said decrease.

7. The method of claim 5 or claim 6, including taking a reading of light reflected from said second surface of said matrix prior to application of said sample to said first surface of said matrix.

8. The method of any one of claims 5 to 7, including collecting and storing a background detector current reading in the absence of reflected light from said illuminating means.

9. The method of any one of claims 1 to 8 wherein each matrix comprises a polyamide.

10. The method of claim 9, wherein the nominal pore size of said matrix is from 0.2 to 1.0 μm.

11. The method of any one of claims 1 to 10, for measuring the concentration of glucose in said whole blood, wherein the signal-producing system comprises 3-methyl-2-benzothiazolinone hydrazone hydrochloride and 3-dimethylamino benzoic acid.

12. The method of claim 11, wherein the signal-producing system is at a pH of 3.8 to 5.

Fig. 1.

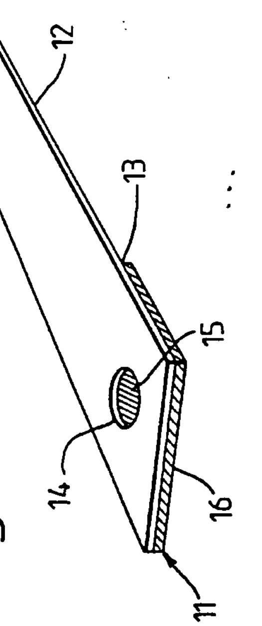

12
13
15
14
16
11

Fig. 2.

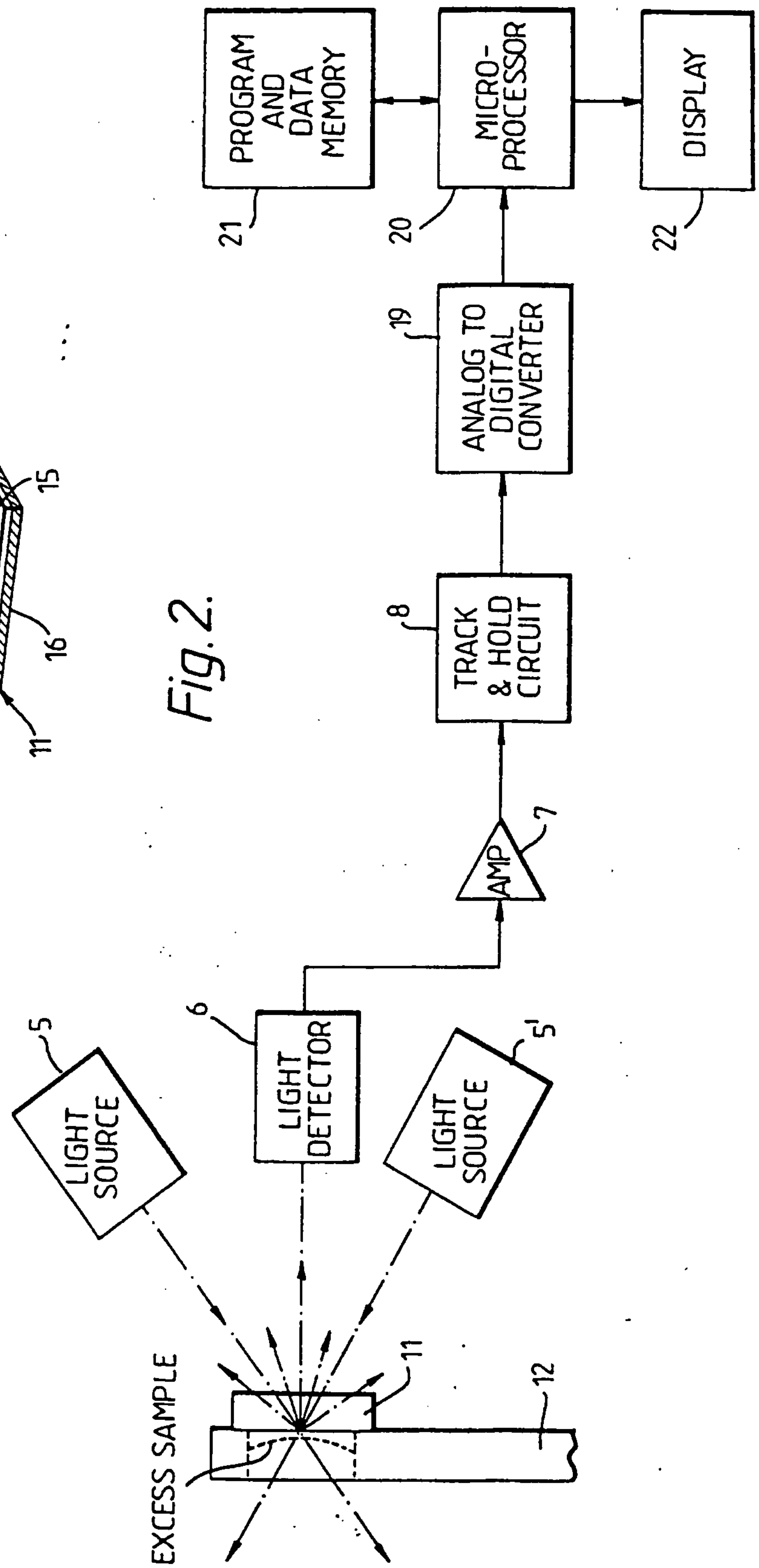
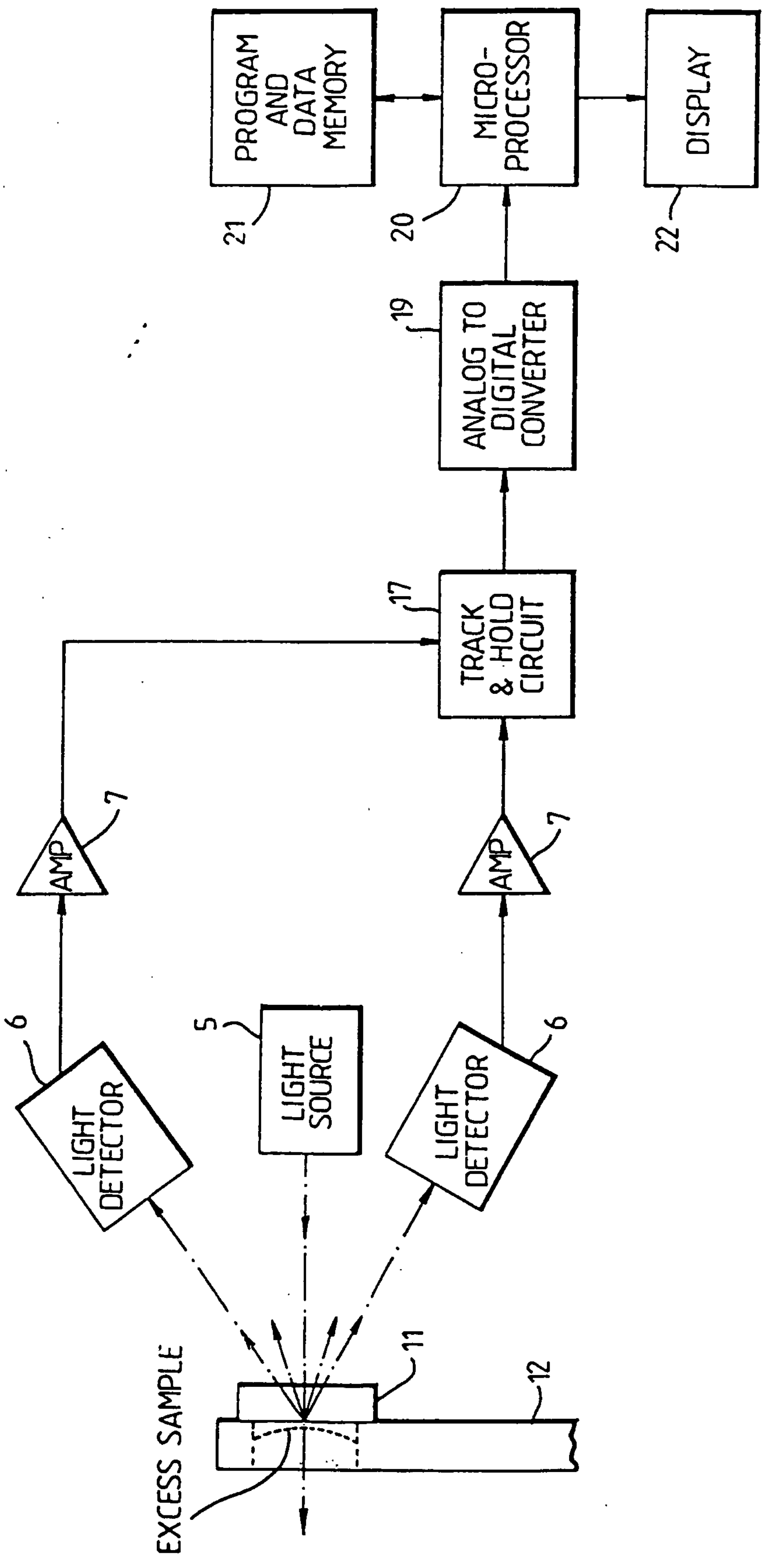

PROGRAM AND DATA MEMORY
21
MICRO-PROCESSOR
20
DISPLAY
22
ANALOG TO DIGITAL CONVERTER
19
TRACK & HOLD CIRCUIT
8
AMP
7
6
LIGHT DETECTOR
5
LIGHT SOURCE
5'
LIGHT SOURCE
EXCESS SAMPLE
11
12

Fig. 3.


PROGRAM AND DATA MEMORY
MICRO-PROCESSOR
DISPLAY
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ANALOG TO DIGITAL CONVERTER
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TRACK & HOLD CIRCUIT
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AMP
7
LIGHT DETECTOR
6
LIGHT SOURCE
5
LIGHT DETECTOR
6
EXCESS SAMPLE
11
12